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Linking life and technology to shape the future

Biosynthesis of plant polyphenolic compounds with therapeutic and industrial relevance

Joana L. Rodrigues, Daniela Gomes, João Rainha, and Ligia R. Rodrigues

Abstract

Polyphenols are naturally produced in plants and have several biological and potential therapeutic activities such as anti-inflammatory, antioxidant and anticancer. They have an estimated market size of USD 2.26 billion by 2027. However, polyphenols are extracted from plants where they accumulate in low amounts over long growth periods. In addition, their purification is difficult and expensive since it requires the separation of other compounds with similar chemical structures in an environmentally unfriendly process. Heterologous microbial production has several benefits as it is not limited by plant availability or environmental factors and it is a renewable, environmentally friendly and sustainable approach. Herein, we report the construction of artificial pathways for the production of curcuminoids and furanocoumarins using *Escherichia coli* as chassis. These compounds can be produced from tyrosine or hydroxycinnamic acids as precursors and have in common the phenylpropanoids pathway. Pure curcumin production from ferulic acid achieved 563 mg/L. Curcuminoids were also produced from tyrosine (42 mg/L) using a modular pathway combining synthetic biologic and co-culture engineering. To our knowledge, these are the highest titers of curcuminoids obtained to date. CRISPR-Cas9 was used to disrupt the *lacZ* gene in order to follow co-culture population composition.

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Curcuminoids Relevance and Pathway

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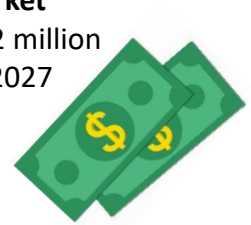
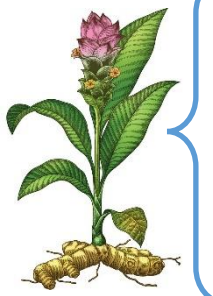
Introduction

Curcuminoids biological activities

- Antioxidant
- Anticancer
- Anti-inflammatory
- Anti-viral
- …

Market
USD 152 million
by 2027



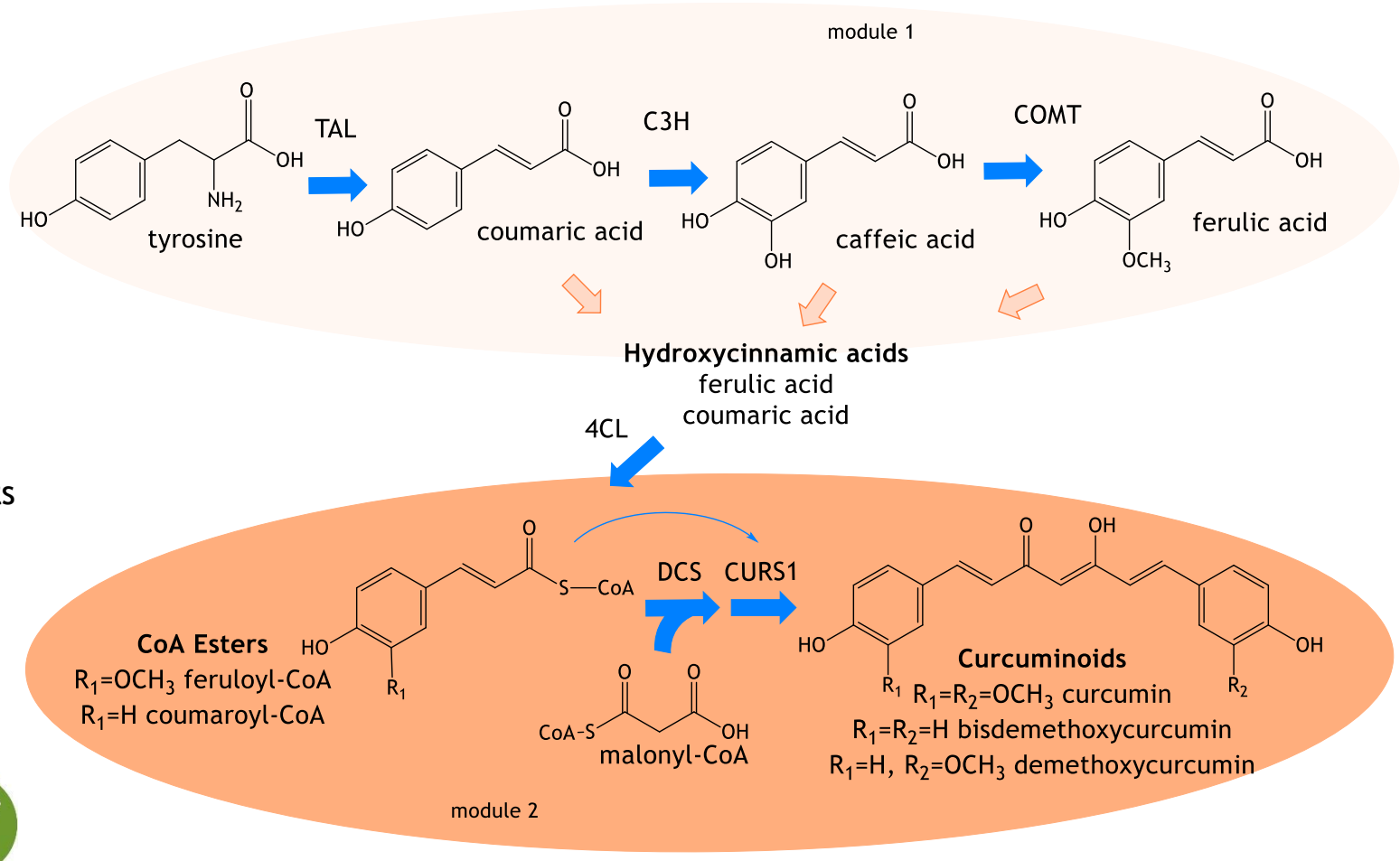


Curcuma longa

- Low amounts, purity and yields
- Limited by seasonality, weather, pests
- Environmentally unfriendly process
- Expensive downstream processes

Combinatorial Biosynthesis

Co-culture strategy



CURCUMINOIDS PRODUCTION IN *E. COLI* USING COMBINATORIAL BIOSYNTHESIS

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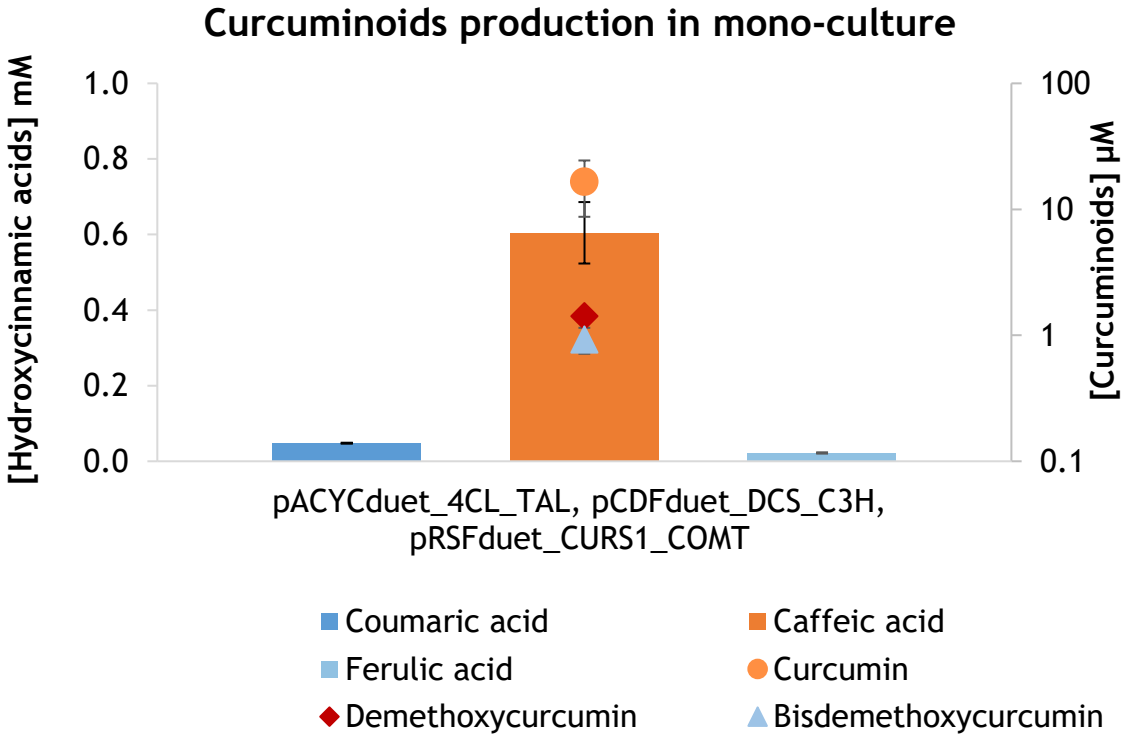
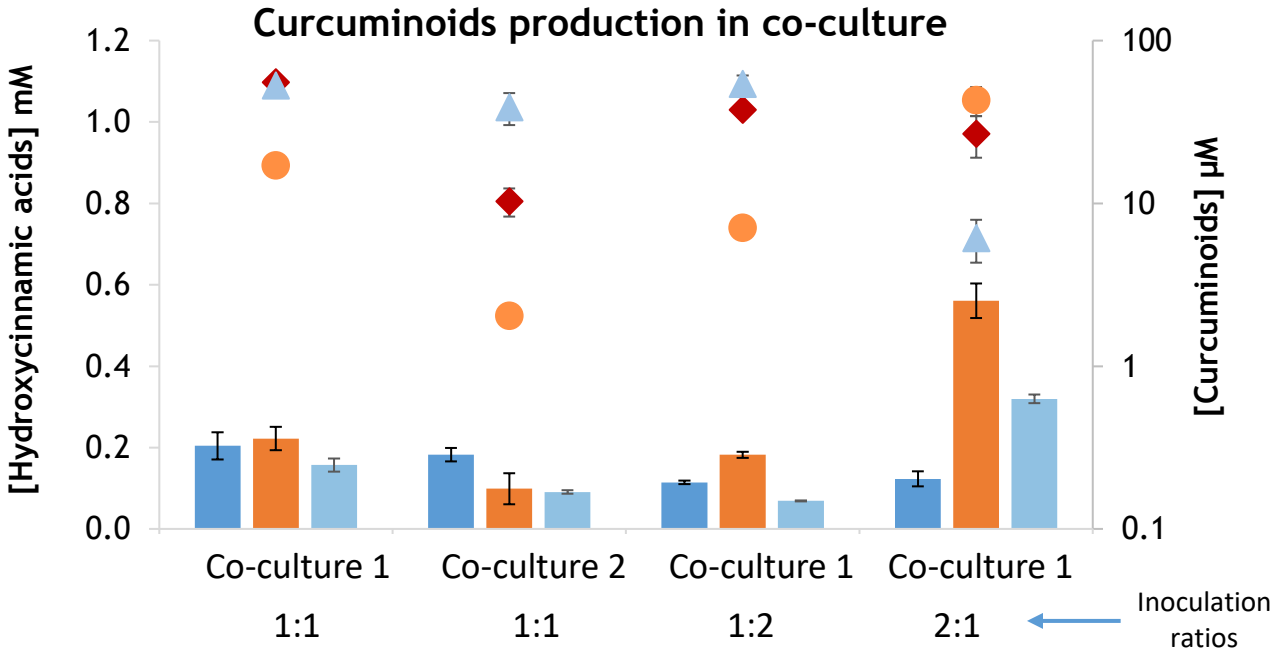
Results





Co-culture 1: Module 1: pCDFduet_TAL + pRSFduet_C3H_COMT;
Module 2: pACYCduet_4CL + pRSFduet_CURS1_DCS

Co-culture 2: Module 1: pRSFduet_TAL_C3H + pACYCduet_COMT;
Module 2: pACYCduet_4CL + pRSFduet_CURS1_DCS

Conclusions

Co-culture allowed to reduce the metabolic burden and increase curcuminoids production

→ Highest productions reported so far

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